Inhibition of fatty acid oxidation activates transforming growth factor-beta in cerebrospinal fluid and decreases spontaneous motor activity.

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Abstract

We have previously reported that transforming growth factor (TGF)-beta in the cerebrospinal fluid (CSF) is involved in the mechanism underlying the regulation of spontaneous motor activity (SMA) by the central nervous system after exercise. However, it remained unclear what physiological condition triggers the activation of TGF-beta. We hypothesized that the shortage of energy derived from fatty acid (FA) oxidation observed in the early phase of exercise activated TGF-beta in the CSF. To test this hypothesis, we investigated whether mercaptoacetate (MA), an inhibitor of FA oxidation, could induce an activation of TGF-beta in the CSF and a decrease in SMA. Intraperitoneal (i.p.) administration of MA activated TGF-beta in CSF in rats and depressed SMA; 2-deoxyglucose, an inhibitor of carbohydrate oxidation, on the other hand, depressed SMA but failed to activate CSF TGF-beta. Intracisternal administration of anti-TGF-beta antibody abolished the depressive effect of MA on SMA. We also found that the depression of SMA and the activation of TGF-beta in the CSF by i.p. MA administration were eliminated by vagotomy. Our data suggest that TGF-beta in the CSF is activated by the inhibition of FA oxidation via the vagus nerve and that this subsequently induces depression of SMA.

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2 Keywords

3 TGF-beta, exercise, fatty acid oxidation, vagotomy, mercaptoacetate, 2-deoxyglucose

4

1. Introduction

We previously demonstrated that transforming growth factor (TGF)-beta in the cerebrospinal fluid (CSF) was activated after exercise and that intracisternal (i.c.) administration of TGF-beta decreased spontaneous motor activity (SMA) in rodents [20,42]. These results indicated that activated TGF-beta in the CSF was one of the components in the system responsible for SMA depression after exercise. However, it remained unclear what physiological condition leads to activation of TGF-beta in the CSF.

The fact that TGF-beta in the CSF is activated during moderate intensity exercise [20] allows us to assume that certain metabolic alterations observed in exercise trigger the activation of TGF-beta in the CSF. During the transition from a sedentary state to the early phase of exercise, several catabolic alterations occur [37] in response to a drastic increase in energy expenditure [21]. Therefore, we focused on energy metabolism in the early phase of exercise, which we regarded as that period from the commencement of exercise until the achievement of a metabolic equilibrium state at a particular exercise intensity, which usually seems to be 10-20 minutes [17,21]. The

main energy supply resources in exercise are carbohydrate and fat [7,8]. Because of the immediate responsiveness of glycogenolysis to increased energy demand [8], carbohydrate oxidation can rapidly increase in the early phase of exercise [17,21]. Fatty acid (FA) oxidation, on the other hand, increases gradually [17], because the responsiveness of FA mobilization from fat depots depends on triacylglycerol lipolysis. Lipolytic activity in adipose tissues during exercise is mediated by the autonomic nervous system and hormones; additionally, the delivery of fatty acids from adipose tissue to working muscles is mediated by increased blood flow velocity, which is also regulated by the autonomic nervous system and hormones [2,15,19]. Energy production from FA oxidation in the early phase of exercise is thus relatively inadequate until FA mobilization reaches a sufficient extent. Indeed, intravenous lipid infusion can increase FA oxidation in the early phase of exercise [33]. In addition, especially in this phase of exercise, FA levels in the blood can decrease [12]. These observations suggest that the extent of FA mobilization in the early phase of exercise is insufficient for the capacity for FA oxidation, or that, in other words, a relative shortage of energy derived from FA oxidation occurs in this phase of exercise. In the normal physiological state, such a

shortage is observed only in the early phase of exercise.

Based on the foregoing, we hypothesized that a relative decrease in FA oxidation can trigger the activation of TGF-beta in the CSF. Indeed, intracisternal administration of TGF-beta induces a decrease in SMA [20] and an increase in FA oxidation [42]. The role of TGF-beta in the CSF is therefore counter-regulatory in response to a relatively decreased FA oxidation level. To test our hypothesis, we investigated SMA and TGF-beta levels in CSF in rats after intraperitoneal (i.p) administration of mercaptoacetate (MA), an FA oxidation inhibitor [3]. Administration of MA can mimic the physiological condition observed as the relative shortage of energy derived from FA oxidation in the early phase of exercise. To compare the effect of inhibition of fatty acid oxidation with that of the inhibition of carbohydrate oxidation, we also analyzed activated CSF TGF-beta concentrations and SMA after i.p. administration of 2-deoxyglucose (2-DG), a carbohydrate oxidation inhibitor [41]. We found that both metabolic inhibitors decreased SMA in rats, although only MA, the FA oxidation inhibitor, activated TGF-beta in the CSF. We also demonstrated that TGF-beta in the CSF is responsible for decreased SMA through the inhibition of FA

oxidation.

2. Materials & Methods

2.1 Animals

Seven-week-old male Sprague-Dawley rats (Oriental Bio Service, Kyoto, Japan) were used. All animals were maintained under controlled environmental conditions (22°C ± 0.5°C, 12-h light-dark cycle), and fed normal chow (MF, Oriental Yeast, Tokyo, Japan) and water ad libitum for at least 1 week prior to their use in experiments. All animals were treated humanely, as outlined in the National Research Council's guide for the Care and Use of Laboratory Animals (Kyoto University Animal Care Committee, according to NIH #86-23, revised 1985). In addition, all procedures were approved by the Kyoto University Animal Care and Use Committee. All experiments were started 2 h after the onset of the dark cycle.

2.2 Determination of spontaneous motor activity after administration of metabolic inhibitors

MA (Wako, Osaka, Japan) and 2-DG (Nakalai Tesque, Kyoto, Japan) were administered i.p. to rats. To determine appropriate experimental conditions, we tested

200, 400, and 600-μmol/kg body weight (B.W.) doses of MA, and 200, 400, and 600-mg/kg B.W. doses of 2-DG. Time-course changes in SMA were measured over 1, 2, and 3-h durations for both MA and 2-DG. We selected doses of 400 μmol/kg B.W. for MA and 400 mg/kg B.W. for 2-DG, and a 2-h duration for all subsequent experiments. MA causes decreased energy production from FA oxidation [28] by inhibiting acyl-CoA dehydrogenase activity [3]. 2-DG induces a decrease in glucose oxidation through the competitive inhibition of phosphohexose isomerase [41]. MA and 2-DG were dissolved in sterile saline (Otsuka Pharmaceutical, Tokyo, Japan), and the same volume of saline was administered i.p. to rats in a control group. The rats were then deprived of food. After 2 h, the rats were moved from their home cage to a test cage (25 × 38 × 17.5 cm). SMA was measured using an infrared ray sensor (Muromachi Kikai, Tokyo, Japan) as described previously [42].

2.3 CSF Collection and determination of the level of active TGF-beta in the CSF

MA (400 μmol/kg B.W.) and 2-DG (400 mg/kg B.W.) were i.p. administered under the conditions described above. Two hours after administration, rats were

anesthetized by administration of urethane (i.p., 1.5 g/kg B.W.) and CSF was collected. CSF collection and TGF-beta level assay were as described previously [27]. Briefly, CSF was collected from the cisterna magna after puncturing the atlantooccipital membrane with a 26G needle fitted to the tip of a micropipette and centrifuged at 2000 *g* at 4°C. The supernatant was then collected and stored at –70°C until TGF-beta assay. TGF-beta concentrations in CSF were determined with a bioassay using TGF-beta-responsive mink lung epithelial cells (TMLCs) constitutively transfected with a TGF-beta-responsive human plasminogen activator inhibitor-1 promoter-luciferase construct (kindly provided by Dr. M. Abe, Department of Nanomedicine, Tokyo Medical and Dental University, Tokyo, Japan, and Dr. D. Rifkin, Department of Cell Biology, NYU Medical Center, New York, NY, USA) [1]. All samples were diluted with Dulbecco's modified Eagle's medium (DMEM) containing 0.1% bovine serum albumin (BSA). TMLCs suspended in DMEM with 10% fetal bovine serum were seeded onto 96-well plates (10,000 cells/well) and allowed to attach for 6 h; thereafter, the medium was replaced with 100 μL of sample solution. After a further 16 h, luciferase activity was measured using a luciferase assay system (Promega, Ann Arbor,

MI), according to the manufacturer's instructions. Levels of active TGF-beta in CSF are expressed as relative luminescence units (RLU). The average RLU value in control groups was defined as 100%.

2.4 Intracisternal administration of anti-TGF-beta antibody

Rats were anesthetized by i.p. injection of 1 mg/kg pentobarbital sodium (Dainippon Seiyaku, Osaka, Japan) and placed in a stereotaxic frame adapted for rat surgery. Subsequently, the skull was exposed and a hole drilled to insert a cannula (24G). Coordinates for placing the cannula in the cisterna magna were as follows: AP, −2.5; ML, 0.0; and DV, −7.9 from the lambda; angle, −32° posteriorly. Rats were allowed 1 week to recover, and only animals with body weights greater than before surgery were used. On the day of an experiment, 5 μg anti-TGF-beta or control preimmune antibodies (R&D Systems, Minneapolis, MN) dissolved in 10 μL artificial cerebrospinal fluid (a-CSF; 140 mM NaCl, 3.35 mM KCl, 1.2 mM Na_2PO_4, 0.3 mM NaH_2PO_4, and 0.10% fatty-acid-free BSA) were administered i.c. Immediately afterwards, MA (400 μmol/kg B.W.) or 2-DG (400 mg/kg B.W.) was i.p. administered.

Saline was administered as a control.

2.5 Vagotomy

After anesthetization with an i.p. injection of 1 mg/kg pentobarbital sodium, rats were vagotomized as described by Li and Anderson [24]. In brief, a 5-cm midline incision was made in the abdomen to expose the stomach and lower esophagus. Thereafter, the bilateral vagal trunks were exposed and cut. A similar procedure was carried out during sham surgery, except that the vagal trunks were not damaged. Rats were allowed 2 weeks to recover from surgery. Because vagotomy decreases food intake for 2 weeks [24], operations were judged successful in rats whose food intake decreased for at least 2 weeks. Rats that failed to regain pre-operation body weight were excluded from subsequent experiments. There was an interval of at least 1 week between each SMA experiment. Firstly, SMA was determined after the i.p. administration of saline, and then the effects of i.p. administration of MA (400 μmol/kg B.W.) and 2-DG (400 mg/kg B.W.) on SMA were determined. Finally, SMA was determined in the untreated state. This sequence of experiment was not randomized, but

we confirmed the last administration of inhibitors did not affect the next basal counts of SMA after one-week interval. CSF was collected from freshly vagotomized rats after a 2 week recovery period, as described above.

2.6 Statistical analysis

All data are expressed as mean ± S.E.M. Data in Figures 1B, 1D, 2, and 5 were analyzed using Student's *t* test. Data in Figure 3A were analyzed using a two-way analysis of variance (ANOVA), and there was a significant interaction between i.c. and i.p. administration. The data were then analyzed using one-way ANOVA, followed by multiple comparison testing using a post-hoc Tukey's multiple comparison test. Data in Figure 3B were analyzed using a two-way ANOVA, and there was no significant interaction between i.c. and i.p. administration. The data were then analyzed using a Student's *t* test in the same factor. Data in Figure 4 were analyzed using a one-way ANOVA, followed by multiple comparison testing using a post-hoc Tukey's multiple comparison test. P values of 5% or less were considered statistically significant. Prism 4 for Mac (GraphPad Software, CA, USA) was used for statistical analysis.

3. Results

3.1 Effect of i.p. administration of MA and 2-DG on SMA

Firstly, we investigated whether the inhibition of FA oxidation depressed SMA similarly to exercise [20] or TGF-beta i.c. administration [20,42]. We carried out SMA measurements after inhibition of FA oxidation by MA i.p. administration. Time-course changes in SMA over 60 min, 2 h after i.p. administration of MA (400 μmol/kg B.W.), are presented in Figure 1A. The cumulative SMA value in the MA group was significantly lower than that in the saline group (Fig. 1B, $P = 0.0036$). To compare the effect of MA with another metabolic inhibitor, we tested whether 2-DG alters SMA in rats. Time-course changes in SMA over 60 min, 2 h after i.p. administration of 2-DG (400 mg/kg B.W.), are presented in Figure 1C. The cumulative SMA value in the 2-DG group was significantly lower than that in the saline group (Fig. 1D, $P < 0.0001$).

3.2 Effect of i.p. administration of MA and 2-DG on CSF TGF-beta levels

To address whether these metabolic inhibitors activate TGF-beta in CSF, we

next measured TGF-beta levels in CSF after MA or 2-DG administration. Two hours after i.p. administration, the TGF-beta level in the CSF of rats given MA (400 μmol/kg B.W.) was significantly higher than that in rats given saline (Fig. 2A, $P = 0.0295$). In contrast, there was no significant difference between TGF-beta levels in the CSF 2 h after i.p. administration in rats given 2-DG (400 mg/kg B.W.) and rats given saline (Fig. 2B, $P = 0.6153$).

3.3 Effect of i.c. administration of anti-TGF-beta antibody on SMA after i.p. administration of MA and 2-DG

To determine whether increased TGF-beta in the CSF is required for decreased SMA after administration of MA, we tested whether i.c. administered anti-TGF-beta antibody eliminated the effect of MA on SMA. Previously, we had demonstrated that i.c. anti-TGF-beta antibody administration completely abolished the physiological effect of TGF-beta in the CSF [18, 19]. There was a statistically significant interaction between i.c. anti-TGF-beta antibody administration and i.p. MA administration (Fig. 3A, $P = 0.026$). The cumulative SMA value over 60 min in the

control group that received preimmune antibody was significantly lower than that in the group that had received the anti-TGF-beta antibody (Fig 3A, $P < 0.05$). There was no significant interaction, however, between i.c. anti-TGF-beta administration and i.p. 2-DG administration (Fig. 3B, $P = 0.2973$). In both the preimmune and anti-TGF-beta antibody-treated groups, cumulative SMA values over 60 min were significantly lower than that in the i.p. saline group (Fig. 3B, preimmune antibody, $P = 0.0339$; anti-TGF-beta, $P = 0.00234$).

3.4 Effect of vagotomy on SMA after i.p. administration of MA and 2-DG

To determine whether the vagus nerve is involved in the effect of MA on SMA, we tested whether vagotomy reverses the depression in SMA after i.p. MA administration. In vagotomized rats, there was no significant difference between cumulative SMA values in the i.p. MA and i.p. saline groups (Fig. 4A, $P > 0.05$). SMA after the i.p. administration of 2-DG was significantly lower than that in the i.p. saline group (Fig. 4A, $P < 0.01$). In sham-operated rats, respective cumulative SMA values for the MA and 2-DG groups were significantly lower than that of the saline group (Fig.

4B, MA, $P < 0.05$; 2-DG, $P < 0.0001$w). In the sedentary state, the cumulative SMA values of vagotomized and sham operated rats were 11030 ± 758 and 15120 ± 381.5, respectively ($P < 0.001$ by Student's *t* test).

3.5 Effect of vagotomy on CSF TGF-beta levels after intraperitoneal administration of MA

Finally, we tested whether vagotomy reversed the increase in TGF-beta produced by i.p. MA administration. In vagotomized rats, there was no significant difference between CSF TGF-beta levels in the i.p. MA and i.p. saline groups (Fig. 5A, $P = 0.371$). In sham-operated rats, the TGF-beta level in the CSF was significantly higher in animals that had received i.p. MA than in those that had received saline (Fig. 5B, $P = 0.0467$). In the untreated state, there was no significant difference between TGF-beta levels in the CSF of vagotomized and sham-operated rats (data not shown).

4. Discussion

In this study, we demonstrated that the FA oxidation inhibitor, MA, activated TGF-beta in the CSF via the vagus nerve. Decreased SMA after exercise might prevent further undesirable energy exhaustion in animals and allow restoration of energy stores. The brain collects peripheral metabolic information and coordinates animal behavior to maintain energy homeostasis [29,36]. Thus, the decrease in SMA after exercise may not only result from the exhaustion of energy stores and potential damage in peripheral tissues but might also reflect a process that is more actively regulated by the CNS. The entire mechanism by which SMA is decreased after exercise by the CNS is still poorly understood; however, our reports [14,20,21,42] together with the results obtained in this study highlight that TGF-beta in the CSF plays a crucial role in the mechanism underlying the regulation of behavior by the CNS after exercise.

It has previously been unclear what physiological conditions trigger the activation of TGF-beta in the CSF during exercise. We focused on the unique metabolic alteration which is observed as a relative shortage of energy derived from FA oxidation in the early phase of exercise [12,33]. We then hypothesized that the shortage of energy

derived from FA oxidation activated TGF-beta in the CSF. As expected, the inhibition of FA oxidation activated TGF-beta in the CSF (Fig. 2). To rule out of the possibility that the activation of TGF-beta in the CSF by inibition of FA oxidation is observed only in MA administration, we measured TGF-beta levels in the CSF after i.p. administration of etomoxir, another inhibitor of FA oxidation. Etomoxir also activated TGF-beta in the CSF (Supplement Figure 1). Additionally a decrease in locomotor activity by administration of etomoxir is already shown [16]. Therefore, the activation of TGF-beta in the CSF and the subsequent decrease in SMA surely result from the inihibtion of FA oxidation. The inhibition of carbohydrate oxidation, on the other hand, did not activate TGF-beta in the CSF (Fig 2). Taking into consideration that i.c. administration of TGF-beta increases FA oxidation [42], TGF-beta in the CSF is involved in a counter-regulatory system that responds to decreased FA oxidation. We showed that i.c. administration of anti-TGF-beta antibody prevented the depressive effect of i.p. administration of MA on SMA (Fig. 3). This indicates that the activation of TGF-beta in the CSF is required for the depression of SMA induced by inhibition of FA oxidation. Taken together, our data suggest that the physiological condition that activates

TGF-beta in the CSF during exercise is likely the shortage of energy derived from FA oxidation observed in the early phase of exercise.

It has been reported that the increased food intake observed following MA administration [35] is eliminated by vagotomy [23]. MA inhibits acyl-CoA dehydrogenase in the liver [3], decreases membrane potential and induces depolarization in hepatocytes [6], and leads to increased afferent activity in the hepatic vagus branch [26]. These observations suggest that information concerning deficient production of energy from FA oxidation is transmitted to the brain via the vagus nerve. Vagotomy eliminated the depressive effect of i.p. administration of MA on SMA (Fig. 4A), but not that of 2-DG (Fig. 4B). Intraperitoneal MA administration to vagotomized rats did not activate TGF-beta in the CSF (Fig. 5A). We are unable to rule out the possibility that ablation of the effect of MA on SMA by vagotomy resulted from the intrinsic effects of the vagotomy itself, which also affected SMA in the sedentary state. However, SMA values (Fig. 4) did appear to be inversely correlated with the concentration of active TGF-beta in CSF (Fig. 5). These data strongly support the contention that the vagus nerve is important in the activation of TGF-beta induced by

inhibition of FA oxidation.

It is unclear how the liver detects changes in FA oxidation levels and transmits signals to the brain via the vagus nerve. Because either administration of fatty acid or inhibitors of fatty acid oxidation, but not glucose, alters plasma membrane potential in hepatic cells *in vitro* and *vivo* [6,26,34], it is possible that efferent sensory neurons directly sense an alternation in fatty acid oxidation. A report that the neuronal sensing pathway of inhibition of FA oxidation is different from that of glucose oxidation [30] also supports this notion. Inhibition of FA oxidation leads to reduction in adenosine triphosphate (ATP)/ adenosine diphosphate (ADP) ratio in the liver [13,18], on the other hand, a single administration of 2-DG does not decrease ATP/ADP ratio in the liver [38]. Then it is another explanation that consequence of reduction in ATP/ADP ratio induced by inhibition of FA oxidation results in changes in hepatic plasma membrane potential, which might be transmitted to sensory neurons by yet-unidentified mechanisms. Further studies are necessary to elucidate how information concerning deficient FA oxidation is transmitted from the liver to the brain.

Despite numerous *in vitro* studies and *in vivo* studies under pathogenic

conditions having shown the mechanism underlying the activation of TGF-beta [5,9,22,44], to the best of our knowledge there is no clear understanding of the TGF-beta activation mechanism in the CSF of rodents under *in vivo* physiological conditions. TGF-beta is widely distributed in the brain [39], and is expressed by various cell types [5]. The TGF-beta receptor is also widely expressed in the brain [4]. Therefore, from an immunohistochemical point of view, the particular location within the brain where TGF-beta is activated and responsible for depressing SMA and enhancing FA oxidation cannot be determined. However, we previously demonstrated that i.c. TGF-beta administration activates hypothalamic noradrenergic neurons [14], which project from the vicinity of the nucleus of the solitary tract (NTS). This suggests that TGF-beta can affect the NTS. Visceral information is transmitted mainly to the NTS via the vagus nerve system [36,40]. Therefore, inhibition of FA oxidation may activate TGF-beta via the vagus nerve and the NTS. Intravenous administration of MA induced c-Fos expression in several region including, NTS, hypothalamus, lateral parabrachial nucleus (IPBN), and the central nucleus of amygdala (CNA). [31]. The lesion of NTS, IPBN and CNA abolish the MA-induces appetites feeding behavior.

[10,30,32]. Additionally, the finding that MA-induced feeding behavior is abolished in decerebrate rats indictates that the projection from NTS to forebrain is important for the feeding behavior induced by the inhibition of FA oxidation [11]. These reports are consistent with our report that TGF-beta in the brain activates NTS-hypothalamus circuits [14]. Further studies are needed to elucidate the mechanism underlying the activation of TGF-beta in the NTS and the subsequent decrease in SMA.

In conclusion, we have demonstrated that i.p. administration of MA, which inhibits FA oxidation, induces decreased SMA in rats. This effect of MA administration is transmitted via the vagus nerve and mediated by TGF-beta in the CSF. Impaired FA oxidation has been reported to lead to decreased SMA [16]. In addition, carnitine is an essential substance for beta-oxidation of FA and a decrease in SMA observed in fasting carnitine-deficient mice [25] can be rescued by carnitine administration [43]. This report also suggests a relationship between a reduction in energy supplied by FA oxidation and a decrease in SMA. Taking other studies into consideration, inhibition of FA oxidation strongly appears to lead to down-regulation of locomotor activity via the liver-vagus nerve-brain system. Our data suggest that TGF-beta in the CSF plays a

crucial role in this mechanism.

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Figure Legends

Fig. 1. Effect of intraperitoneal (i.p.) administration of energy metabolism inhibitors [mercaptoacetate (MA) and 2-deoxyglucose (2-DG)] on spontaneous motor activity (SMA). SMA over 60 minutes was determined 2 h after i.p. administration of MA (400 μmol/kg B.W.; A and C) and 2-DG (400 mg/kg B.W.; B and D). An identical volume of saline was administered to animals in the control group. (A) and (B) show time course changes in SMA. (C) and (D) show cumulative SMA values from 0 to 60 min. Values are mean ± S.E.M. (n = 13-14). (***, $P < 0.001$; **, $P < 0.01$ by Student's *t* test)

Fig. 2. Effect of i.p. administration of energy metabolism inhibitors on transforming growth factor-beta (TGF-beta) levels in the cerebrospinal fluid (CSF). CSF was collected 2 h after i.p. administration of MA (400 μmol/kg B.W.; A) or 2-DG (400 mg/kg B.W.; B). An identical volume of saline was administered to animals in the control group. The average relative luminescence units (RLU) value in the control group was defined as 100%. Values are mean ± S.E.M. (n = 12-14). (*, $P < 0.05$ by

Student's t-test)

Fig. 3. Effect of intracisternal (i.c.) administration of anti-TGF-beta or non-immune control antibodies (5 μg dissolved in 10 μL) on SMA after i.p. administration of energy metabolism inhibitors MA (400 μmol/kg B.W.; A) and 2-DG (400 mg/kg B.W.; B). An identical volume of saline was administered i.p. to control animals. SMA cumulative values over 1 h were determined 2 h after the i.c. and i.p. administrations. Values are mean ± S.E.M. (n = 6-8). (**, $P < 0.01$; *, $P < 0.05$ by two-way ANOVA, followed by one-way ANOVA and post-hoc Tukey's multiple comparison test (A) and two-way ANOVA followed by Student's *t* test to identify which group differences accounted for the significant P value (B))

Fig. 4. Effect of vagotomy on SMA after i.p. administration of the energy metabolism inhibitors MA (400 μmol/kg B.W.; A) and 2-DG (400 mg/kg B.W.; B). An identical volume of saline was administered i.p. to control animals. SMA cumulative values over 1 h were determined 2 h after i.p. administration. Values are mean ± S.E.M. (n = 7-8).

(**, P < 0.01; *, P < 0.05 by one-way ANOVA, followed by a post-hoc Tukey's multiple comparison test.)

Fig. 5. Effect of vagotomy (A) and sham surgery (B) on TGF-beta levels in the CSF 2 h after i.p. administration of MA (400 μmol/kg B.W.). An identical volume of saline was administered to animals in the control group. The average RLU value in control groups was defined as 100%. Values are mean ± S.E.M. (n = 12-14). (*, P < 0.05 by Student's *t* test)

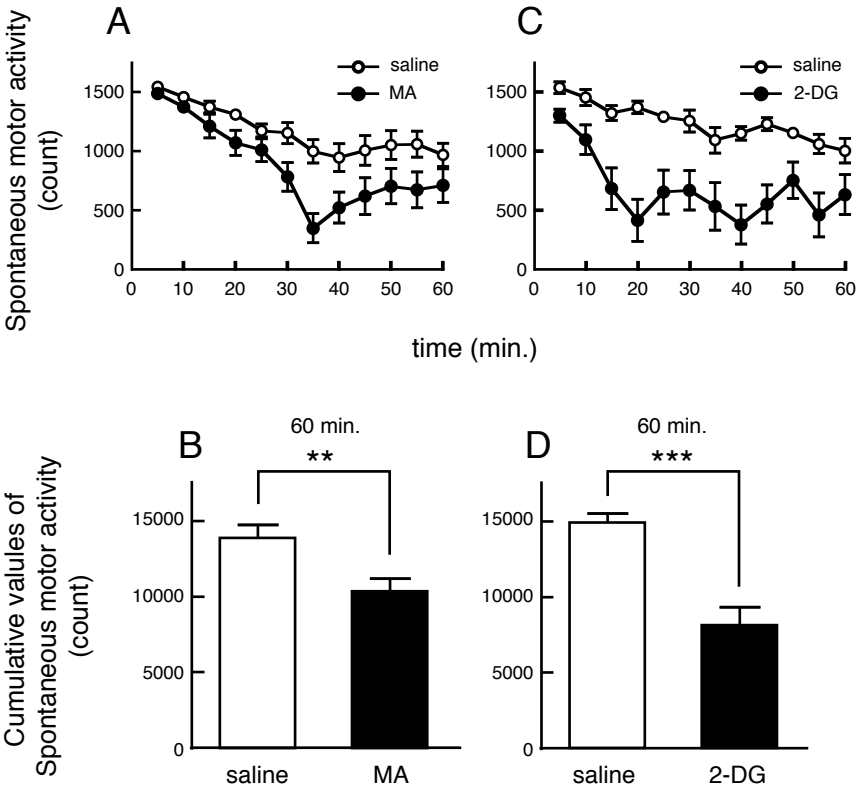


Figure 1

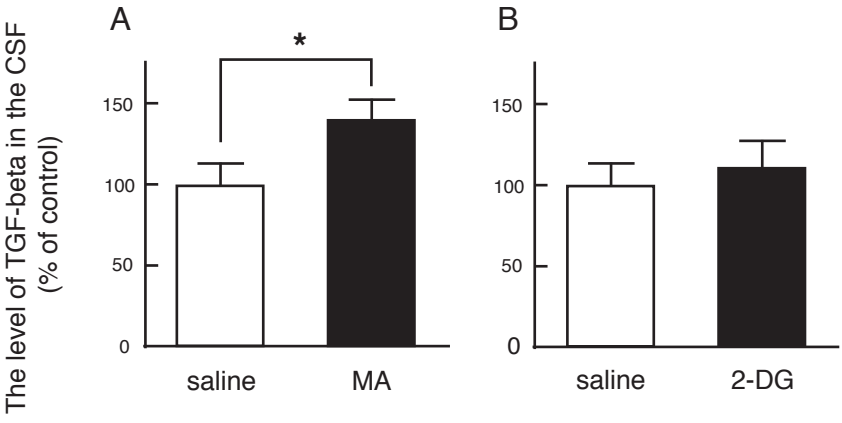


Figure 2

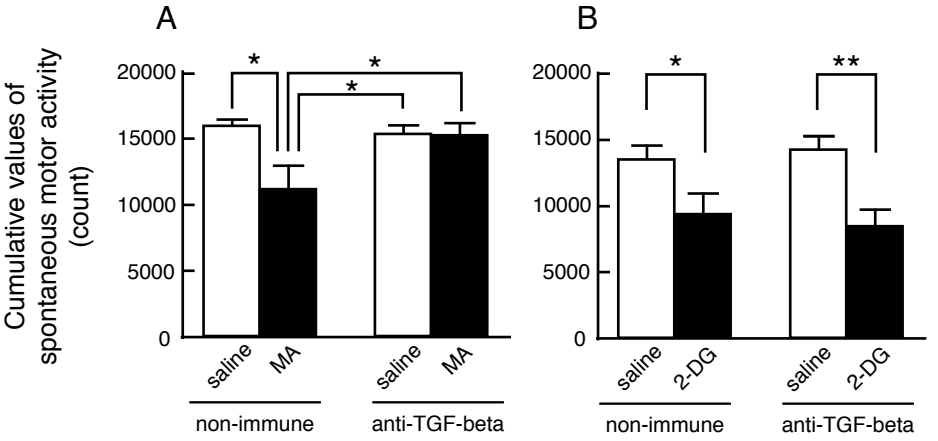


Figure 3

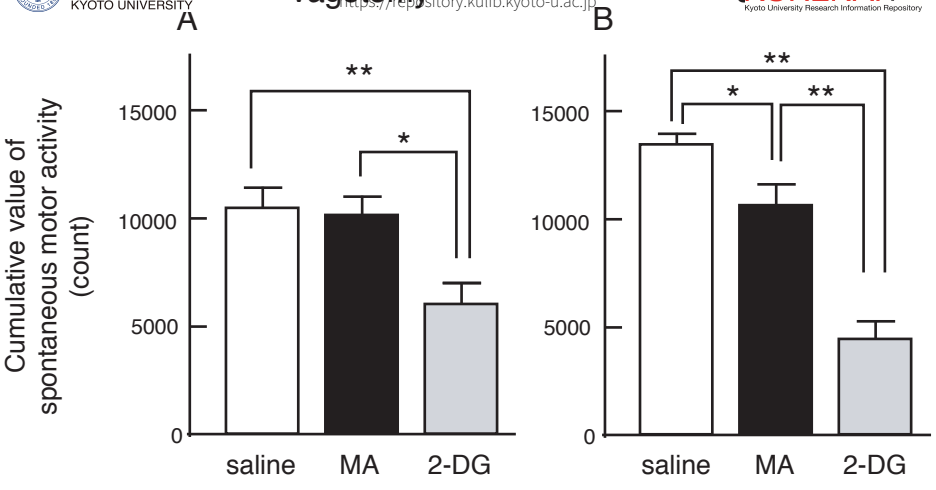


Figure 4

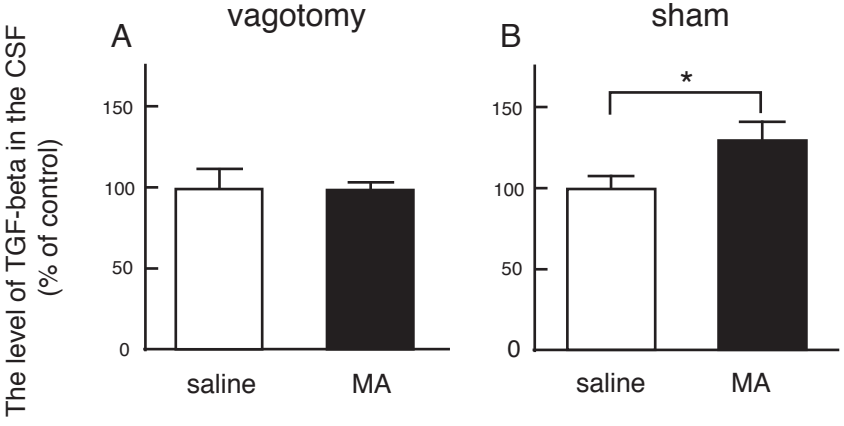
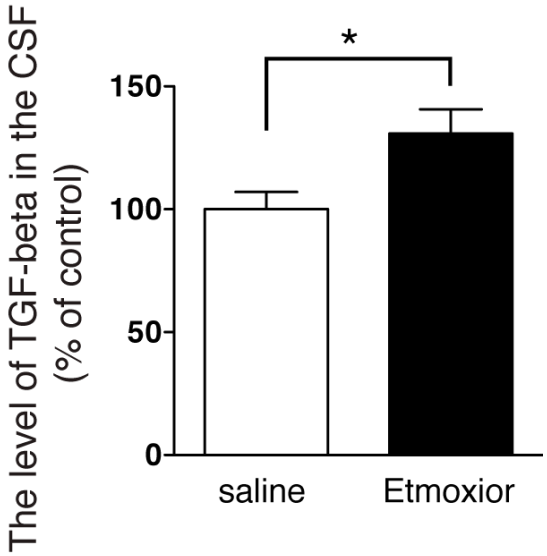


Figure 5

Supplementary Figure 1



Legend

Supplementary Figure 1. Effect of i.p. administration of etomoxior on TGF-beta level in the CSF. CSF was collected 2 h after i.p. administration of etomoxior (25 mg/kg B.W.) An identical volume of saline was administered to animals in the control group. The average relative luminescence units (RLU) value in the control group was defined as 100%. Values are mean ± S.E.M. (n = 6-7). (*, $P < 0.05$ by Student's t-test)

Materials & Methods

Etomxior was purchased from Sigma Aldrich Japan (Tokyo, Japan). Etomoxir was dissolved in sterile saline (Otsuka Pharmaceutical, Tokyo, Japan). Saline was used as control. Other protocols are described in Materials & Methods section in article.